Elucidating the Role of Dopamine Receptor D4 in Cartilage

Tarek Obeid[1], Scott Gronowicz [1], Janapriya Vijayakumar [1], Justine Tigno-Aranjuez[2], Thomas J. Kean[1]
[1]Biionix Cluster, Internal Medicine, College of Medicine, University of Central Florida, Orlando, FL, USA
[2]Burnett School of Biomedical Sciences, University of Central Florida, Orlando, FL, USA
ta515256@ucf.edu

Disclosures: T.J. Kean: 4; ABT, AZN, FATE, FUJIY, GILD, GWW, MRK, VSTM. 9; Innovation Network Committee Member, Cartilage Topic Co-Chair.

INTRODUCTION: Osteoarthritis (OA) is a widespread and debilitating disease. Current therapies for osteoarthritis are primarily palliative and there is a strong need to identify novel therapeutics to prevent the progression of OA. In a recent natural product screen, aromoline was identified as being able to stimulate type II collagen expression[1]. Aromoline is a bisbenzylisoquinoline alkaloid that showed upregulation of its target protein, dopamine receptor D4 (DRD4), and type II collagen[1]. The identification of DRD4 in cartilage was a novel finding but its role is unknown. In this study, we sought to use CRISPR/Cas9 to knockout DRD4 and investigate its role in chondrogenesis using primary human chondrocytes and the chondrocyte cell line, TC28A2.

METHODS: Immortalized human chondrocytes (TC28A2, Sigma-Aldrich) and primary human chondrocytes isolated from a 4-day-old neonatal femur (IIAM) were cultured and maintained. To confirm the expression of DRD4 in the cells, immunocytochemistry and qPCR were performed. Immunostaining was carried out using an anti-DRD4 clone 2B9 mouse monoclonal antibody (Millipore Corp MABN125) and Hoechst 33342 (Invitrogen H1399) for nuclear staining. Fluorescence microscopy (Keyence BZ-X810) was used to visualize stained cells. To validate the presence of DRD4 at the RNA level, qPCR was performed with HPRT as the reference gene. A lentiviral vector, LentiCRISPRv2, was used for the knockout experiments with a guide targeting DRD4 and a negative guide (sigma) control (pLCP). Lentiviral particles were applied to the cells with polybrene. Growth media was replaced with media containing puromycin to select for infected cells. The DRD4 knockout IIAM and TC28A2 cells were subjected to immunocytochemistry staining to confirm the loss of DRD4 expression. Additionally, qPCR was performed to further verify the knockout. The effect of DRD4 knockout on chondrogenesis in 3D aggregate culture is currently being assessed to determine whether DRD4 affects aggregate formation and ECM accumulation.

RESULTS: Immunocytochemistry (**Fig. 1**) and qPCR confirmed successful knockout of the DRD4 gene in IIAM and TC28A2 cells after transduction with the DRD4 KO LentiCRISPRv2 plasmid. Immunocytochemistry staining shows the DRD4 protein in red. In the initial images captured before knockout, both IIAM and TC28A2 cells exhibited the characteristic red fluorescence indicating the presence of DRD4. Comparing the images captured before and after knockout, a significant difference is observed. Additionally, qPCR data showed no amplification for the target gene (DRD4) in knockout cells compared to the control (pLCP) and untreated. We are currently performing aggregate growth, TGFβ1 dose response, biochemical, and histological analyses in order to determine the effects of DRD4 knockout on chondrogenesis.

DISCUSSION: In this study, we explored the role of dopamine receptor D4 (DRD4) in chondrogenesis. DRD4 is a novel G-protein expressed in human chondrocytes and a potential therapeutic target for osteoarthritis (OA). The lack of druggable G-protein-coupled receptors (GPCRs) in cartilage underlies the dearth of anabolic solutions to OA. Notably, the potential linkage between DRD4 and the transforming growth factor beta (TGF-β) pathway suggests intricate regulatory interactions. This successful confirmation of DRD4 knockout will be used to investigate aromoline's mechanism of action in elevating type II collagen expression, bolstering DRD4's candidacy as a therapeutic target. Collectively, these findings emphasize the need for deeper exploration of the intricate molecular networks connecting DRD4, TGF-β signaling, and cartilage health, opening novel avenues for innovative interventions with disease-modifying potential in osteoarthritis management.

SIGNIFICANCE/CLINICAL RELEVANCE: The identification of dopamine receptor D4 (DRD4) as a potential therapeutic target for osteoarthritis, its novel connection to the transforming growth factor beta (TGF-β) pathway, and its influence on chondrogenesis suggest promising avenues for innovative disease-modifying interventions with the potential to transform OA management and improve patient outcomes.

REFERENCES:

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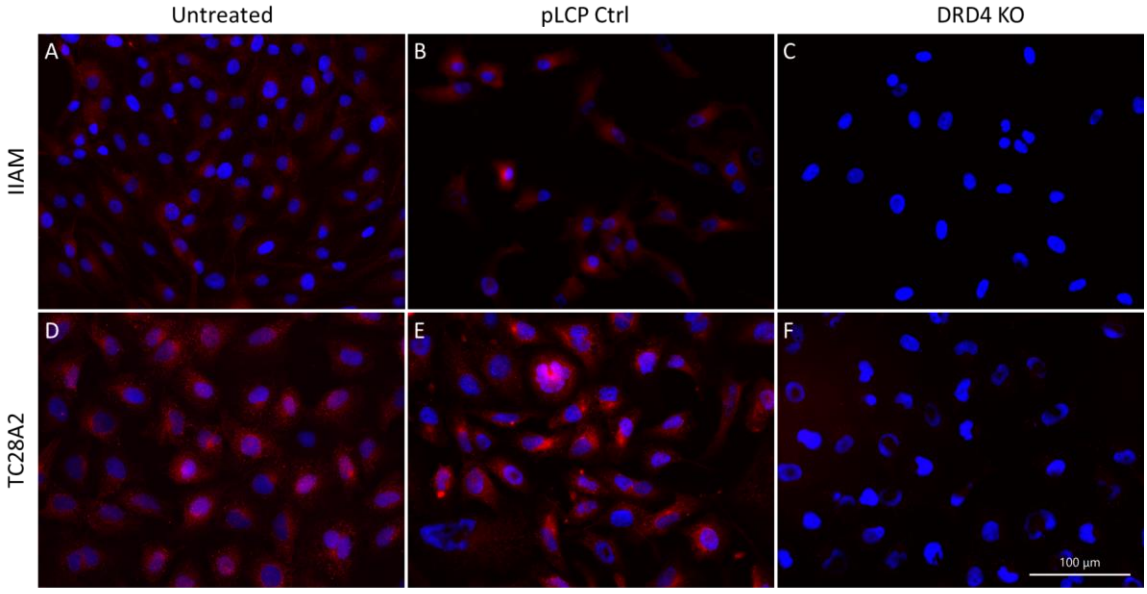

Figure 1: Untreated cells = A,D; CRISPR lentiviral control, pLCP Ctrl = B, E; CRISPR DRD4 knockout, DRD4 KO = C,F. IIAM cells A-C; TC28A2 cells D-F. D4 (DRD4) expression shown in red (anti-DRD4 antibody), cell nuclei in blue (Hoechst 33342), Scale bar = 100 µm, same magnification used for all images.